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Short communication

Border Disease in persistently infected calves: Radiologic and pathologic findings.

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Border disease (BD) virus belongs to the genus *Pestivirus* together with bovine viral diarrhoea virus genotypes 1 and 2 (BVDV-1 and -2) and classical swine fever virus (CSFV) (Pletnev and others 2011). The pathogenicity of BD virus for bovine fetuses has been recognised early (Gibbons and others 1974), but only since 1994 renewed attention has been drawn to the mutual transmission between species (Carlsson and Belák 1994). Transmission of BD virus from persistently infected sheep to cattle was demonstrated (Krametter-Frötscher and others 2008; Krametter-Frötscher and others 2009; Reichle 2009; Braun and others 2013a; Braun and others 2013b). Nevertheless, reports on clinical disease in cattle caused by BD virus are rare (Cranwell and others 2007; Krametter-Frötscher and others 2010; McFadden and others 2012).

The present report describes the radiologic and pathologic findings in three calves (Table 1) persistently infected with BD virus. Persistent infection with pestivirus was suspected based on an initial positive ear notch sample taken within one week after birth on the three different farms of origin and tested in private laboratories by ELISA or RT-PCR. This was later confirmed in our lab using real-time RT-PCR and serology on blood samples collected independently on at least two occasions, a minimum of 17 days apart. Thus, all calves were strongly positive for viral RNA and negative for serum antibodies against pestiviruses at the latest time point analysed, i.e. at the age of 2, 8, and 15 month after birth for animal 1, 2, and 3, respectively. This identifies all three calves as persistently infected with a pestivirus. Genotyping based on the sequence within the 5' untranslated region (5'-UTR) of the pestiviral genome (Bachofen and others 2008) revealed that calves were persistently infected with BD virus 'Switzerland' (Peterhans and others 2010). This was corroborated by the facts that (i) the mother animals of all three calves possessed significantly higher neutralising antibodies against BD virus than BVDV as assessed by serum neutralisation tests (SNT), and that (ii) sheep were kept on all three farms from where the PI animals originated. For further diagnostic investigation, immunohistochemistry using monoclonal antibodies against virus antigens

was carried out on snap-frozen skin biopsy specimens (calf 1 to 3; Ca3/34-C42, specific for BVDV and antibody C16, specific for pestivirus) as described (Hilbe and others 2007). The staining with pestivirus-specific antibodies was positive and with BVDV-specific ones negative in all of the three calves, in accordance with BD virus infections.

Calf 1 had a moderate body condition and appeared stocky, calves 2 and 3 were in good body condition with a normal demeanor at the time of admission. Radiographic evaluation of calf 2 was undertaken 52 days after admission because of right hind limb lameness and swelling of the lateral aspect of the right stifle. Radiographs showed bands of increased radiopacity in the proximal tibial and distal femoral metaphyses.

Consequently radiographs of the left metacarpus and metatarsus were taken in all three calves. Radiographic examination was repeated 5 months after the initial examination in calf 2. Computed tomographic (CT) examination of the left fore- and hindlimbs was carried out in calf 1 and calf 2 (Table 1). CT in calf 2 was carried out under general anaesthesia, in calf 1 at post-mortem since it had to be euthanised because of enteritis, bronchopneumonia and omphalitis. The calves were scanned in lateral recumbency using 120 kVp, 200 mA and the pictures evaluated in a bone and soft tissue window.

Radiographs in calf 1 (Fig. 1) showed concentric radiopaque rings in the carpal and tarsal bones as well as in the epiphyses of the radius, tibia, metacarpal and metatarsal bones and phalanges. The metaphyses of the long bones had multiple, alternating, parallel bands of high and low radiopacity. The medullary radiopacity of the bones was increased but was homogeneous without apparent structure, the cortex was thickened and the diameter of the bones appeared reduced (Fig. 1 a). Computed tomography revealed generalized severe trabecular sclerosis of all examined bones. The carpal, tarsal and phalangeal bones had concentric zones of greatly increased attenuation (Fig. 1 b). The radiologic diagnosis was chronic, severe, generalised recurrent osteopetrosis with secondary moderate abnormalities of the shape and structure of the long bones.

Radiographs in calf 2 showed parallel transverse bands of increased radiopacity in the metaphyses of the metacarpi, -tarsi and digits (Fig. 2 a, c) and irregular, increased radiopacity of the medullary cavity of the metacarpal / metatarsal bones. The trabecular sclerosis in all bones as well as alterations in the shape and structure of the cortex of long bones were less pronounced overall in the images of the computed tomography than those seen on radiographs. In calf 2, radiographs taken at the age of eight months exhibited milder changes than at the age of three months (Fig. 2 b, d). The banding in the metaphyses was replaced with inhomogeneous moderate sclerosis, indicating regressive osteopetrosis.

The radiographic findings in calf 3 consisted of a mild increase in radiopacity of the diaphyses which extended to the metaphyses. The metaphyseal vascular channels were accentuated by neighbouring sclerosis, indicating mild osteopetrosis.

In necropsy the left fore- and hind limb of calf 1 were cut in the transverse plane revealing mostly striate, but sometimes circular white dense bone structures alternating with normal bone ('bone-in-bone') in the epiphyses and metaphyses of the humerus, radius, ulna, os femoris, tibia, metacarpal and metatarsal bones and phalanges (Fig. 2 c). After gross examination, the bones were sawed into small pieces, fixed in formalin for 24 hours and then decalcified with EDTA-citrate acid for 4 weeks. Histological examination of the white areas yielded a marked increase in small, plump, retained primary trabeculae (osteopetrosis) that were interwoven transversely and longitudinally and formed the so-called 'growth arrest lines'. Between these structures were slender, partly interwoven, normal primary trabeculae, which were slightly decreased in number (mild osteoporosis). There were a scant number of osteoblasts and even fewer osteoclasts visible. Immunohistochemistry using monoclonal antibodies against the virus antigen E^{rns} (C42, specific for BVDV and 15C5, specific for pestiviruses) was carried out on paraffin-embedded material of various organs (Hilbe and others 2007) and on decalcified bone material (Hilbe and others 2000; Nuss and others 2005). All samples were positive with pestiviruses-specific but negative with BVDV-specific antibodies. In bone mate-

rial, pestivirus antigen was visible multifocally in osteocytes.

The present cases document, to our knowledge for the first time, radiologic and pathologic changes in bone structure in three calves born persistently infected with BD virus after natural, transient infections of their mother animals, analogous to changes described in aborted bovine fetuses persistently infected with BD virus (Gibbons et al., 1974) and bovine fetuses and calves persistently infected with BVDV (Constable and others 1993; O'Connor and Doige 1993; Scruggs and others 1995; Hilbe and others 2000; Nuss and others 2005; Smirnova and others 2008; Webb and others 2012). The severity of clinical and radiologic changes varied with the individual calves; the calf with the most severe clinical signs also had the most severe radiologic and histologic lesions. Similar to radiographic lesions in a calf persistently infected with BVDV (Nuss and others 2005), clinical and radiographic signs improved with age in calf 2, and were hardly visible in calf 3, which was radiographed at the age of 16 months. This indicates that persistent infection with BD and BVD virus in calves can basically be diagnosed radiographically provided that radiographs are taken in the first few weeks of life, when changes are still distinct. Radiographic and pathologic changes in calves persistently infected with BD virus are not readily distinguishable from lesions in calves persistently infected with BVDV. Therefore, both viruses should be considered equally pathogenic for cattle.

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201 Table

202

203

204 Table 1 Data of calves persistently infected with BD virus at the time of radiographic exami-

205 nations.

Nr.	Breed	Age at 1st/2nd radiographic examination (months)	Height at the withers at 1st/2nd radiographic examination	Weight at 1st/2nd radiographic examination	Age at CT examination (months)	Age at pathologic examination (months)
1	Swiss Braunvieh	4	76 cm	63 kg	5	5
2	Swiss Braunvieh	3/8	89/104 cm	92/153 kg	4.5	n.d. (calf still alive)
3	Swiss Braunvieh-Limousin cross	16	n.d.	358 kg	n.d.	n.d.

206 n.d.: not done

207 Figure legends

208

209

210 Fig. 1 Diagnostic imaging and pathomorphologic preparation of the left forelimb of calf 1
211 with severe skeletal changes a) Orthogonal radiographic views of the left forelimb at 4
212 months of age show concentric rings of increased radiopacity in the carpal bones and metaph-
213 yses and epiphyses of the metacarpus, cortical thickening and medullary sclerosis, and mild
214 dorsal deviation of the digital axis (asterisk) b) Dorsal reconstruction of computed tomo-
215 graphic images taken at 5 months of age (green lines indicate sections transverse to the longi-
216 tudinal axis) show the concentric layers of increased attenuation ('bone-in-bone', white ar-
217 rowheads), the thickening of the cortex at the level of mid-diaphysis (white arrows) and the
218 sclerosis of the medullary cavity (black arrowheads) c) Longitudinal section of the left fore-
219 limb of calf 1 at 19 weeks of age in the transverse plane showing compacted bone structures
220 with a white to beige circular appearance (carpal bones, 'bone-in-bone' appearance) or with
221 an elongated dense white to beige appearance near the epiphysis and in the metaphysis of the
222 metacarpal bone. The normal colour of the bone and medullary cavity is red.

223

224 Fig. 2 Plantarodorsal (a) and lateromedial (c) radiographic views of the metatarsal bone of the
225 left hind limb of calf 2 at three months showing mild patchy sclerosis of the distal metaphysis,
226 which is interrupted by transverse radiolucent zones (white arrows), and mild cortical
227 thickening of the diaphysis (white arrowheads). Corresponding radiographic findings at the
228 age of eight months are shown in b) and d); the changes are less pronounced than at three
229 months. There is increased radiodensity of the medullary cavity and the diaphyseal cortex
230 (black arrowheads). There is reorganisation of the banded structure of the metaphysis seen at
231 three months of age and a moderate irregular sclerosis of the metaphyseal trabeculae.